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MANNITOL IN SERUM OF HEMODIALYSIS PATIENTS MEASURED BY FLUOROMETRY AND MANNITOL DEHYDROGENASE FROM LEUCONOSTOC MESENEROIDES, E.P. Diamandis, L.C. Grass, R. Uldall, D. Mendelsohn, D. Maini (Departments of Clin. Biochem. and Int. Med. The Toronto Hosp. and Univ. of Toronto, Toronto, Canada)

Mannitol is now frequently used intravenously during modern hemodialysis to abolish the problems of hypotension and dialysis disequilibrium syndrome. We have evaluated two chemical methods and an HPLC procedure for measuring mannitol in serum. The chemical methods were based on the oxidation of mannitol by periodate and measuring the liberated formaldehyde with either chromotropic acid (spectrophotometry) or acetylacetone (fluorometry). These three procedures were found unsuitable for routine use either for non-specificity (chemical methods) or impracticality (HPLC). We thus developed a new enzymatic fluorometric procedure which is based on the oxidation of mannitol by β -NAD⁺ in the presence of mannitol dehydrogenase (MD) to fructose and NADH. Our method involves deproteinization of serum with trichloroacetic acid. Forty μ L of protein-free supernatant were then mixed with 30 μ L of a Tris buffer containing β NAD⁺ (10 mg/mL) and 10 μ L of a 10 U/L MD solution. After incubation for 1 h at room temperature, the mixture was diluted with 2 mL of a Tris buffer and NADH fluorescence was measured with ex-em of 340 - 450 nm. Blanks were run for all samples by eliminating the enzyme addition. The enzyme MD was purified from cultures of *leuconostoc mesenteroides*. The assay was applied for measuring mannitol in normal serum and sera from hemodialysis patients who never received the sugar and gave negative results in all cases. Mannitol levels in the serum of hemodialysis patients who received mannitol, correlated well with the amount of administered dose in all cases. Measuring range was from 25-500 mg/L; detection limit 10 mg/L; recovery between 82-100% (mean 92%) and precision 4.7% (within-run) and 9.8% (between-run) within the measuring range. This assay is simple to perform and has the advantage of being very specific, eliminating the glucose and glycerol interference of chemical methods. It is now used to monitor mannitol administration in hemodialysis patients.